PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P1006PC00		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/DK02/00083	06/02/2002	09/02/2001
International Patent Classification (IPC) or national classification and IPC C12N9/99		
Applicant CHR. HANSEN A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/09/2002	03.04.2003
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Giebeler, K Telephone No. +49 89 2399 8546

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/DK02/00083

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-15	as originally filed				

Claims, No.:

1-32	as received on	30/11/2002	with letter of	28/11/2002	

Drawings, sheets:

1/2,2/2	as originally filed				

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   ☐ the language of publication of the international application (under Rule 48.3(b)).
   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.
   ☐ filed together with the international application in computer readable form.
   ☐ furnished subsequently to this Authority in written form.
   ☐ furnished subsequently to this Authority in computer readable form.
   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:
   ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)
   see separate sheet

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	
	No:	Claims	1-9,13-18
Inventive step (IS)	Yes:	Claims	19-32
	No:	Claims	10-12
Industrial applicability (IA)	Yes:	Claims	1-32
	No:	Claims	

2. Citations and explanations
   see separate sheet

Re Item I

Basis of the report

1. The amendments filed with the letter dated 28.11.02 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendment concerned is the introduction of the term "pH of less than 1.9" into claim 1(ii). No basis could be found in the application as filed for this pH range, and no such basis has been indicated by the applicant.

   Consequently, this report has been established on the basis of claims 1-32 as originally filed, claims 2-32 as originally filed being identical to claims 2-32 as filed with letter dated 28.11.02. Claims 33 and 34 have been deleted by the applicant.

Re Item V

Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

2. The following documents are cited:

   D1: WO 97 20921 A
   D3: US-A-3 950 221

3. The present application does not satisfy the criterion set forth in Article 33(1)(2) PCT because the subject-matter of claims 1-9 and 13-18 is not new.

   The document D1 discloses methods for the selective inactivation of enzyme activities using acidic or alkaline conditions. Example 6 describes the inactivation of undesired cellulases at pH 2.0. This method is not clearly distinguished from that of claim 1 which refers to "a pH of less than 2.0" and includes pH values of 1.99 (see claims 13-15). Consequently, claims 1-9 and 13-18 are considered to lack novelty.

4. The subject-matter of claims 19-32 is considered to involve an inventive step over the closest prior art document D3 which also relates to a method of improving the

quality of an aspartic protease preparation. It could not be expected from any of the available prior art that the acid treatment according to the claimed method would reduce the content of undesired enzymatic activities while most of the protease activity is retained.

5. Claims 10-12 are considered to lack an inventive step since a person skilled in the art would have known that the methods described in D1 would also be generally applicable to other cultivated organisms. The applicant's argument that the claimed invention involves an inventive step since D1 does not claim removal of e.g. glucoamylase could not be followed since claims 10-12 do not refer to glucoamylase as a mandatory feature, either.

6. This authority considers that claims 1-18 lack enablement (Article 5 PCT) and support by the description (Article 6 PCT) to the extent that they relate to polypeptide preparations other than aspartic acid proteases.

Chr. Hansen A/S
PCT/DK02/00083
Our ref: P1006PC00

CLAIMS AFTER RESPONSE TO 1ST WRITTEN OPINION

1. A method of providing a polypeptide preparation having a reduced content of undesired enzymatic side activities, the method comprising the steps of:

(i) providing a medium having a pH of 2.0 or higher that comprises at least one desired polypeptide and in addition hereto at least one undesired enzymatic side activity, and

(ii) subjecting said medium to a pH of less than 1.9 for a period of time that is sufficient to at least partially inactivate the at least one enzymatic side activity.

2. A method according to claim 1 wherein at least 75% of the activity of the at least one desired polypeptide is retained after subjecting the medium having a pH of 2.0 or more to a pH of less than 2.0.

3. A method according to claim 2 wherein at least 85% of the activity of the at least one desired polypeptide is retained.

4. A method according to any of claims 1-3 wherein at least 50% of the activity of the at least one undesired enzymatic activity is inactivated.

5. A method according to claim 4 wherein at least 90% of the activity of the at least one undesired enzymatic activity is inactivated.

6. A method according to any of claims 1-5 wherein the medium having a pH of 2.0 or higher is a medium derived from the cultivation of an organism that during its cultivation produces the at least one desired polypeptide and the at least one undesired enzymatic side activity.

7. A method according to any of claims 1-6 wherein the at least one desired polypeptide is selected from the group consisting of an enzyme, an antibody, an antigen and a pharmaceutically active polypeptide.

8. A method according to any of claims 1-7 wherein the at least one enzymatic side activity is selected from the group consisting of glucoamylase activity, starch degrading enzyme activity, protease activity, peptidase activity, phosphatase activity, lipase activity, cellulase activity, lactase activity and hemicellulase activity.

9. A method according to any of claims 1-8 wherein the medium having a pH of 2.0 or higher is derived from the cultivation of an organism that is selected from the group consisting of an animal species, a plant species, a bacterial species, a yeast species and a species of filamentous fungi.

10. A method according to claim 9 wherein the bacterial species is selected from the group consisting of a gram negative bacterial species including *E. coli* and a gram positive species including a *Bacillus* species.

11. A method according to claim 9 wherein the yeast species is selected from the group consisting of *Saccharomyces cerevisiae*, a methylotrophic yeast species including *Pichia pastoris* and a *Klyuveromyces* species including *Klyuveromyces lactis*.

12. A method according to claim 9 wherein the species of filamentous fungi is selected from the group consisting of an *Aspergillus* species, a *Cryphonectria* species, a *Fusarium* species, a *Rhizomucor* species and a *Trichoderma* species.

13. A method according to any of claims 1-12 wherein the medium having a pH of 2.0 or higher is subjected to a pH in the range of 1.0 to 1.99.

14. A method according to claim 13 wherein the pH is in the range of 1.5 to 1.99.

15. A method according to claim 14 wherein the pH is in the range of 1.7 to 1.99.

16. A method according to claim 15 wherein the pH is about 1.8.

17. A method according to any of claims 13-16 wherein the pH in the range of 1.0 to 1.99 is provided by adding an inorganic or an organic acid.

18. A method according to any of claims 1-17 wherein the medium having a pH of 2.0 or higher is subjected to a pH of less than 2.0 for a period of time that is in the range of 0.1 minutes to 48 hours.

19. A method according to any of claims 1-18 wherein the at least one desired polypeptide has aspartic protease activity.

20. A method according to claim 19 wherein the medium having a pH of 2.0 or higher is a medium derived from the cultivation of a microorganism that during the cultivation produces the aspartic protease and the at least one undesired enzymatic side activity.

21. A method according to claim 20 wherein the medium is derived from the cultivation of a microorganism that naturally produces the aspartic protease or from the cultivation of a recombinant microorganism that has an inserted gene expressing the aspartic protease.

22. A method according to claim 21 wherein the microorganism is selected from the group consisting of a bacterial species, a yeast species and a species of filamentous fungi.

23. A method according to claim 22 wherein the aspartic protease is expressed as a fusion protein having, in addition to the aspartic protease activity, at least one undesired enzymatic side activity.

24. A method according to claim 23 wherein the at least one enzymatic side activity is starch degrading enzyme activity including an activity selected from the group consisting of amylase activity and glucoamylase activity.

25. A method according to any of claims 20-24 wherein the microorganism is one that naturally produces at least one enzymatic side activity.

26. A method according to claim 25 wherein the at least one enzymatic side activity is selected from the group consisting of glucoamylase activity, lactase activity, starch degrading enzyme activity, protease activity, peptidase activity, phosphatase activity, lipase activity, cellulase activity and hemicellulase activity.

27. A method according to any of claims 19-26 wherein the aspartic protease is derived from the group consisting of an animal aspartic protease including a mammalian aspartic protease, a plant aspartic protease and a microbial aspartic protease.

28. A method according to claim 27 wherein the mammalian aspartic protease is selected from the group consisting of pro-chymosin, chymosin, pepsinogen and pepsin.

29. A method according to claim 28 wherein the aspartic protease is derived from a mammalian species selected from the group consisting of a ruminant species, a *Camelidae* species including *Camelus dromedarius*, a porcine species, an *Equidae* species and a primate species.

30. A method according to claim 29 wherein the ruminant species is selected from the group consisting of a bovine species, an ovine species, a caprine species, a deer species, a buffalo species, an antelope species and a giraffe species.

31. A method according to any of claims 27-30 wherein the mammalian derived aspartic protease is a protease naturally produced in a mammalian species.

32. A method according to claim 27 wherein the aspartic protease is derived from a naturally produced aspartic protease by the addition or deletion of one or more amino acids or substitution of one or more amino acids herein.